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THE VANADIUM AND SELECTED METAL CONTENTS OF SOME ASCIDIANS

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The concentration of vanadium and other metals from sea water by animals called ascidians has been known since the turn of the century. Several papers have summarized the information available on the subject (Webb, 1939; Bertrand, 1950; Carlisle, 1968). This paper reports the vanadium and other selected transition metal contents of ascidians primarily from the northern California coast. It has been suggested that the ability to concentrate vanadium to levels greater than the combined levels of other transition metals is confined to certain families in the order Phlebobranchia (Webb, 1939; Carlisle, 1968), but several workers have reported finding substantial concentrations of vanadium in species from the order Aplousobranchia (Goldberg, McBlair and Taylor, 1951; Trason, 1957; Levine, 1961; Ciereszko, Ciereszko, Harris and Lane, 1963). Studies were carried out on certain species from the order Phlebobranchia and Aplousobranchia to determine vanadium content. In addition, since large iron contents have been reported (Endean, 1955) for species from the order Stolidobranchia, observations were made on a species from this order. Electron spin resonance studies were carried out to determine the oxidation states of vanadium in various species. Preliminary spectra are reported for the cells contained in the fluids of *Ascidia ceratodes* and the fractions chromatographed from these cells.

Materials and Methods

Species were collected at the following locations in the spring and summer of 1972 and the spring and summer of 1973: Bodega Bay, *Ascidia ceratodes;* Bodega Marine Laboratory, University of California, *Eudistoma psammion* and *Eudistoma ritteri;* Berkeley Yacht Harbor, *Ciona intestinalis* and *Molgula manhattensis;* Hopkins Marine Station, Pacific Grove, *Amaroucium californicum, A. solidum, Clavelina huntsmani, Cystodytes lobatus, Distaplia occidentalis,* species of genus *Eudistoma, Polyclinum planum* and *Synoicum par-fustis. Rhopalaca abdominalis,* a Caribbean species collected south of Portobello, Panama by Dr. Charles Birkeland, was supplied as a preserved sample by Professor Donald P. Abbott, Hopkins Marine Station. The samples collected at Hopkins Marine Station were identified by Professor Donald P. Abbott. Other samples were identified by comparison with descriptions in standard references (Van Name, 1945; Ricketts, Calvin and Hedgpeth, 1968).

Each sample was cleaned of extraneous material and colonial ascidians were cut to minimize the contamination of the sample by sand. Solitary ascidians were brushed clean and washed. The samples were weighed wet, after drying at 110° C and after ashing at 500–600° C to constant weight. The ashes of several colonial ascidians were redissolved in aqua regia, any remaining particulate matter was re-

moved by filtration and the samples were redried. Such a procedure has no effect on the vanadium analyses. However, there was a direct correlation between the spectrographically determined silicon content of the sample and the iron content. This may result from either the inclusion of sand or ingested diatoms, some of which are known to concentrate iron, in the ascidians. Therefore iron contents are reported only for those samples that have low silicon contents.

The spectrographic analyses were run on a Bausch and Lomb Large Littrow-type Quartz Prism Spectrograph. To insure a proper arc both samples and standards were mixed 1 : 1 by weight with a buffer consisting of (by weight) 50% graphite, 25% $Al_2(SO_4)_3$, and 25% K_2SO_4. Prior to use the graphite electrodes were refluxed in concentrated nitric acid for 2 days. The spectra were recorded on Eastman SA-1 plates. The plates were read with an Applied Research Laboratory Model No. 42 Densitometer. The following lines were used for quantitative determinations of the elements: vanadium, 2526.22 Å, 2530.18, 2923.62, 2924.03, 2924.64, 3183.41, 3183.98 and 3185.39; titanium, 3186.45; chromium, 2677.16 and 3015 (3 lines); iron, 2689.21, 2714.87, 2719.03, 2720.91, 2723.57, 2724.9 (2 lines) 2733.58 and 2735.48; and silicon, 2519.21 and 2528.54. The concentrations of the metals were determined by comparison of the observed density of the line after correction for background with a series of standards. Spectrophotometric analyses using a Cary Model 14 Spectrophotometer were performed for vanadium using the phosphotungstate (Wright and Mellon, 1937) and N-benzoyl-o-tolylhydroxylamine methods (Majumdar and Bhowal, 1971) using ammonium metavanadate as a standard. Iron analyses were performed using the thiocyanate method (Sandell, 1959). The approximate pHs of the samples were determined with universal pH indicator paper. Electron spin resonance (e.s.r.) spectra were recorded at 77° K on a Varian Model E-4 Spectrometer. Order of magnitude concentrations of vanadium (IV) were determined by comparison of the intensities of the spectra with standard solutions of known concentrations run at 77° K.

Spectra of the cells from *Ascidia ceratodes* were taken by allowing them to adhere to the window of a quartz cell and then the spectra were recorded with a Cary Model 14 spectrophotometer equipped with a Model 1471250 High Intensity Light Source. A separation of the contents of the cells in the fluid from *Ascidia ceratodes* using column chromatography is reported. The separation was carried out on CPG-10–175 Controlled-Pore glass beads coated with Carbowax, mesh size 80/120, manufactured by Electro-Nucleonics Inc., using a thermostatable column manufactured by Pharmacia Fine Chemicals, Uppsala, Sweden. Oxygen was purged from the elutent, 0.1 M H_2SO_4, with nitrogen, which had been purified by passing the gas through chromium(II) bubblers. The samples were collected, 4-cc per collection, with a Gilson Automatic Collector and care was taken to flush the collection tubes with nitrogen to minimize oxygen oxidation of the samples. The sample to be eluted was prepared by the following steps: (1) the fluid was centrifuged and the plasma removed; (2) a minimal quantity of 0.1 M H_2SO_4 was added to the cells and the cells were lysed and (3) the residue was centrifuged away from the green-yellow solution. All operations were performed in a nitrogen atmosphere to minimize air oxidation. Protein analyses were made with Folin reagent.

TABLE I

Transition metal analyses of ascidians

Species and systematic position	ppm (org dry wt)[a]		wet weight, g	dry wt % of wet wt.	ash wt % of wet wt.	Comments
	V	Fe				
PHLEBOBRANCHIA						
ASCIDIIDAE						
Ascidia ceratodes						
whole animal	1300	1900	8.46	7.80	2.72	—
blood (ash)	6200	500				[V] ≈ 3–4 × 10^{-3} M,[c] acid, no e.p.r. signal
outer fluid (ash)	8000	500				
fluid[b]	—	—	—	—	—	[V] ≃ 2 × 10^{-3} M
CIONIDAE						
Ciona intestinalis						
blood (ash)	160	50	—	—	—	[V] ≈ 7 × 10^{-5} M[c] Mn 10 ppm
centrifuged blood cells	—	—	—	—	—	[V(IV)] ≈ 3 × 10^{-4} M[d]
PEROPHORIDAE						
Perophora annectens						
whole animal, 1972	8000, 9000[b]	940	4.85	7.24	3.51	acid, no e.p.r. signal
whole animal, 1973	700–2000[b]	—	—	—	—	
DIAZONIDAE						
Rhopalaea abdominalis						
whole animal, preserved	1800	—	5.88	3.97	2.17	
APLOUSOBRANCHIA						
SYNOICIDAE						
Amaroucium californicum (ash)	<10	[e]	3.04	—	5.34	
A. solidum (ash)	<10	~1000	4.78	—	2.94	
Polyclinum planum (ash)	<10	[e]	10.82	10.80	7.55	
Synoicum par-fustis	~10	600	18.81	6.20	2.90	
POLYCITORIDAE						
Clavelina huntsmani	30	900	19.36	3.91	2.15	—
Cystodytes lobatus	50	[e]	5.18	—	6.48	—
Distaplia occidentalis whole colony						
1. orange-yellow	600	1200	2.17	6.0	2.8	acid, pH ~1
2. light green-brown-gray	1200	2200	2.96	6.0	3.0	acid, pH ~1
3. brown red-light brown	600	500	2.79	6.0	2.6	acid, pH ~1
4. brown-white-light brown	1200	2200	3.00	6.0	3.0	acid, pH ~1
Eudistoma diaphanes whole colony	25	[e]	4.97	6.60	2.90	acid, pH ≤2

TABLE 1—*Continued*

Species and systematic position	ppm (org dry wt)[a]		wet weight, g	dry wt % of wet wt.	ash wt % of wet wt.	Comments
	V	Fe				
E. molle						
whole colony surface including zooids	1000	400	2.90	5.90	2.20	acid, pH ≤2
zooids	560–1200[h]	—	—	—	—	—
zooids (ash)	6000, 5000[b]	—	—	—	—	—
test (ash)	1000[b]	—	—	—	—	—
E. psammion						
whole colony	80	[e]	13.28	16.5	11.6	acid, pH ≤2
whole colony	130[b], 130[h]	—	11.30	6.82	3.06	—
zooids	610[b], 614[h]	—	0.160	17.60	3.39	—
E. ritteri						
large whole colony (ash)	320	2600	1.06	—	3.54	Cr, 50 ppm; Ti, ~150 ppm
small whole colony (ash)	270	2400	0.41	—	4.72	Cr, 60 ppm; Ti, ~100 ppm
STOLIDOBRANCHIA						
MOLGULIDAE						
Molgula manhattensis						
animal without tunic-intestines	—	1600[f]	1.19	5.00	2.51	
cleared	—	900[f]	1.67	4.09	2.07	
	—	1200[f]	1.25	4.10	2.19	
	—	1700[f]	1.17	3.95	2.20	
whole animal without tunic-fluid removed from heart-intestines cleared	—	1300[f]	0.716	4.98	2.14	
fluid from heart-intestines cleared	—	8000[f]	0.2015[g]	3.34	2.68	
	—	5000[f]	0.2365[g]	3.60	2.43	
fluid from heart-freshly collected	—	5700[f]	0.2435[g]	3.30	2.25	
animal without tunic-freshly collected	<20	16,400	2.21	6.21	3.60	
	—	17,500[f]	2.02	6.49	3.52	
whole animal	—	26,600[f]	3.17	5.92	3.67	
	<20	—	3.67	6.0	3.0	

[a] ppm (parts per million) organic dry weight unless otherwise specified (*e.g.*, ash-indicates ppm ash weight). Organic dry weight = dry weight—ash weight.

[b] Vanadium determined spectrophotometrically using phosphotungstate method.

[c] "Vanadium concentration" determined from analysis of a known volume of fluid.

[d] Vanadium (IV) concentration estimated using e.s.r. standardized against a 1×10^{-3} M vanadium (IV) in 0.5 M $HClO_4$ at 77° K.

[e] Analysis for Fe unreliable due to presence of particulate matter.

[f] Analysis for Fe using thiocyanate method.

[g] Wet weight is weight of fluid used.

[h] Vanadium determined spectrophotometrically using o-benzoyl-o-tolylhydroxylamine.

Results and Discussion

Observations on species from the order Phlebobranchia

Table I contains data on the vanadium, iron and selected transition metal contents of various ascidians. The species are arranged alphabetically within the orders Phlebobranchia, Aplousobranchia and Stolidobranchia respectively. The species *Ascidia ceratodes* and *Perophora annectens* in the families Ascidiidae and Perophoridae of the order Phlebobranchia have large vanadium contents. These observations are consistent with analyses performed on other species in these two families (species, ppm V in whole animal based on organic dry weight): *Ascidia corelloides*, 1130; *A. nigra*, 3100; *A. mentula* forma *cylindrica* Harant, 470, 1400; *A. mentula* forma *typica* O. F. Muller, 420, 610, 982; *A. aspersa* forma *cristata*, 690, 790; *Phallusia mamillata*, 1200–1300, 1700; *Perophora viridis*, 760; *Ecteinascidia conklini*, 1800; and *E. turbinata*, 100 (work of Webb 1939; Bertrand, 1950; Ciereszko, Ciereszko, Harris and Lane, 1963; Carlisle, 1968).

It should be noted that vanadium analyses of *Perophora annectens* varied between 700–2000 ppm organic dry weight in the spring and summer of 1973 and 8000–9000 ppm in the spring and summer of 1972. It may be that the difference can be ascribed to the maturity of the samples, but no specific study was carried out on this interesting subject (see discussions of *Endistoma molle*, *E. psammion* and *E. ritteri*). Samples of *Ascidia ceratodes* collected during both periods had identical vanadium contents in their fluids. Known volumes of the fluids from the heart and the region just inside the tunic of *Ascidia* ceratodes were reduced to ash, analyzed and "vanadium concentrations" of the fluids were calculated to be between $2–4 \times 10^{-3}$ M. The homogenized fluids of both *Ascidia ceratodes* and *Perophora annectens* were acidic and initially showed no electron spin resonance (e.s.r.) spectrum at 77° K. At later stages the e.s.r. spectrum of vanadium (IV) developed. These observations are consistent with those made on fluids present in the species *Phallusia mamillata* (Boeri and Ehrenberg, 1954; Bielig, Bayer, Dell, Rohns, Mollinger and Rüdiger, 1966), and *Ascidia obliqua* (Boeri and Ehrenberg, 1954; Lybing, 1954), where it is suggested that vanadium (III) is present. It should be noted that in the case of *Ascidia ceratodes* the iron content of the whole animal is considerably higher than the vanadium content, while in the fluids the vanadium content is at least ten times that of iron. *Ciona intestinalis* also shows the property that the blood cells contain a large excess of vanadium over iron while the metal content of the whole animal shows the reverse (Bielig, Pfleger, Rummel and Seifen, 1961). The surface of the tunic of *Ascidia ceratodes* was carefully cleaned to eliminate extraneous matter as a source of iron.

A chromatographic separation of the contents of the cells contained in the fluid of *A. certaodes* was carried out using the methods described under Materials and Methods. Figure 1 represents the spectral characteristics of the dominant species collected and of the cells in a plasma-saline solution. The number of the fraction is used to label a particular spectrum. Fraction 26 and those having the same spectral characteristics contained all of the vanadium in the sample. The vanadium content of a control sample containing the same volume of fluid was 9.8×10^{-3} millimoles, while 9.9×10^{-3} millimoles were accounted for by analysis of the vanadium in fractions 26–29. The spectrum of fraction 26 can be reproduced by a combination of the spectra of vanadium (III) and vanadium (IV) in 0.1 M H_2SO_4.

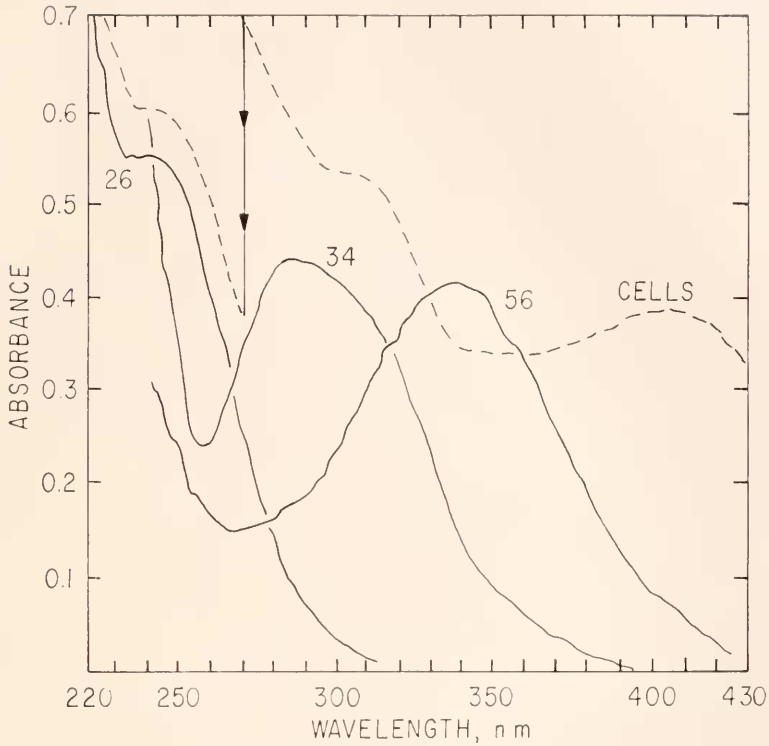

FIGURE 1. Spectra of chromatographed fractions and cells of *Ascidia ceratodes*.

and no spectral evidence is found for a ligand or protein bound to the vanadium. A vanadium (III)-vandium (IV) mixture in 0.1 M H_2SO_4 was passed through the column under the same conditions as the sample and it has the same fraction distribution as the sample. Electron spin resonance (e.s.r.) and spectral studies show some vanadium (IV) is present in the eluted fractions. Therefore it appears that once the cells are lysed some oxidation of vanadium (III) occurred even though precautions were taken to exclude oxygen. Samples eluted without the precaution of removing oxygen showed more vanadium (IV) than those where oxygen was excluded. We feel a possible role of vanadium (III) in the cells is the production of acid by means of an oxidation-reduction cycle.

Using Folin reagent the protein contents of various fractions were (fraction number: μg/ml protein): 26:5, 33:19, 34:33, 38:7 and 56:0. The amount of protein seems to vary with the absorbance represented by fraction 34, which is approximately the absorbance maximum of tryptophan, which has been shown to be present in the protein of *Phallusia mamillata* (Bielig, Bayer, Dell, Rohns, Mollinger and Rüdiger, 1966).

Fraction 56 contains a compound with absorbance maxima at 335 and 205 nm. Aqueous solutions containing this compound (although not shown to be pure) obey Beer's law and are oxidized by vanadium (V) and oxygen. It is not clear what role this species plays in the *in vivo* system, but the possibility is being

investigated that cells containing this compound are involved in the reduction of vanadium (V), the oxidation state of vanadium present in sea water, to the vanadium (III) present in the cells.

As shown in the spectrum of the cells, the components represented by fractions 26 and 34 can account for the shorter wavelength region, but the species represented by fraction 56 appears to be shifted to long wavelengths. Ethanol solutions of fraction 56 are shifted to longer wavelengths and the cell spectrum may reflect the environment of the species in fraction 56.

The vanadium content determined for a preserved sample of *Rhopalaca abdominalis* (1800 ppm organic dry weight) was comparable to that determined for *Rhopalaca neapolitana*, 1700, 2000 ppm organic dry weight (Ciereszko, Ciereszko, Harris and Lane, 1963). The preservation fluid also contained some vanadium suggesting that the metal can be leached from the animal. Thus the value of 1800 ppm is a lower limit on the vanadium content of *Rhopalaca abdominalis*.

The vanadium content of *Ciona intestinalis* is in agreement with the observations of other workers (Goldberg, McBlair and Taylor, 1951; Bielig, Pfleger, Rummel and Seifen, 1961; Carlisle, 1968). The vanadium and iron contents of the blood cells have been determined to be 1500 and 800 ppm dry weight respectively (Bielig, Pfleger, Rummel and Seifen, 1961). The values determined in this study of 160 and 50 ppm ash weight for vanadium and iron in the blood seem reasonable. The manganese content of blood is less than 10 ppm. The high level of manganese found by Carlisle (1968) must result from localization of the metal in other areas of the animal. The 77° K electron spin resonance spectrum of cells centrifuged from the blood of *Ciona intestinalis* shows the presence of vanadium (IV). If the intensity of the e.s.r. spectrum is compared with those of standard solutions and the resulting vanadium (IV) concentration is compared with the vanadium content obtained by direct spectrographic analyses of the blood, it appears that a substantial amount of the vanadium in the blood is present as vanadium (IV) or in a vanadium oxidation state which is readily oxidizable to vanadium (IV).

Observations on species from the order Aplousobranchia

Large vanadium contents have normally been associated with species of certain families of the order Phlebobranchia. However, large vanadium contents and acidic conditions have been found in certain species in the order Aplousobranchia: *Euherdmania claviformis, Amaroucium pellucidum, Eudistoma olivaceum, Eudistoma ritteri, Clavelina picta*, and *Pycnoclavella stanleyi* (work of Goldberg, McBlair and Taylor, 1951; Trason, 1957; Levine, 1961; Ciereszko, Ciereszko, Harris and Lane, 1963). Our investigation shows that certain species in the genera *Distaplia* and *Eudistoma* in the family Polycitoridae from the order Aplousobranchia concentrate large amounts of vanadium. All of the species were acidic, pH ≤ 2. A high level of sulfuric acid in the tunic fluid of *E. ritteri* was observed by Levine (1961). The species with large vanadium contents were *Distaplia occidentalis* (four separate color variations) and some species in the genus *Eudistoma: E. molle* and *E. psammion*. *E. molle* and *E. psammion* were examined to determine the location of the vanadium (zooids or distributed throughout the entire colony). The orange zooids of *Eudistoma molle* were carefully removed from the animal and both the zooids and test were analyzed for vanadium. On the basis of concentrations in ppm ash weight at least 80% of the vanadium present is localized

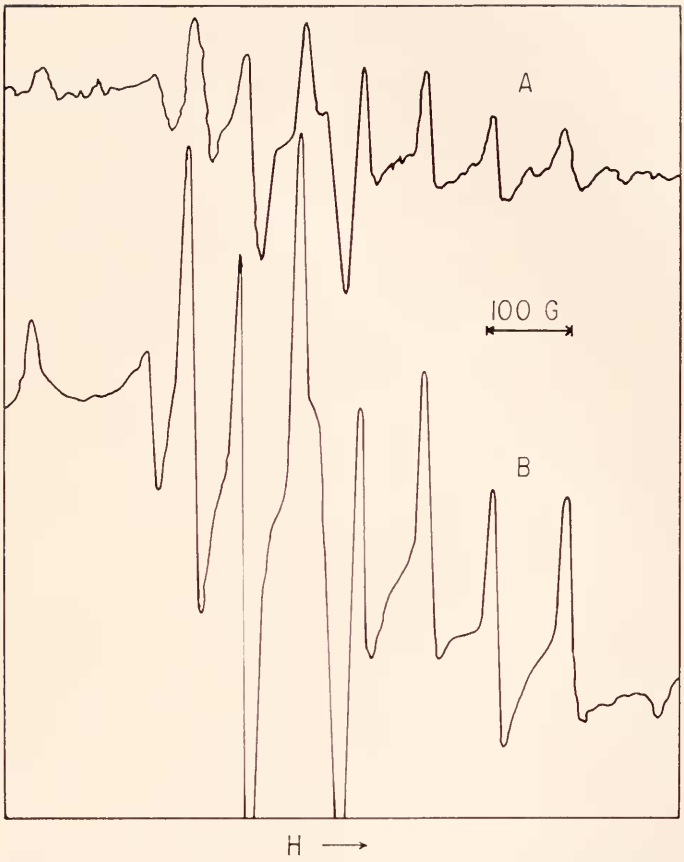

FIGURE 2. Electron spin resonance spectra at 77° K of *Eudistoma diaphanes* (A) and 10^{-2} M VO^{2+} in aqueous acid (B).

in the zooids. Some variation occurred in the vanadium contents of zooids of samples collected in 1972 and 1973, and it is believed that less mature animals contained less vanadium in the zooids. The vanadium in *E. psammion* is also localized in the zooids and analyses in 1972 and 1973 showed identical vanadium contents for whole animals and zooids. The low vanadium content determined for *E. diaphanes*, 25 ppm organic dry weight, may result from the low density of zooids in this species compared to other species of this genus. Levine (1961) has determined the metal content of the zooids from *E. ritteri* and has found the following metals in ppm dry weight: Ti, 1512; V, 471 and Cr, 144. Our analyses in ppm ash weight of *whole animals* show vanadium and chromium at the expected levels, but titanium is an order of magnitude below the level reported by Levine. We have observed variable levels of some metals in *E. ritteri*, for example, some very immature colonies showed a lower vanadium content than more developed colonies. It is possible that animals at various stages of development require different metals, and consequently some discrepancies in metal analyses appear. The metal content

of the marine environment just before and/or at the time of sampling may be important.

The question of the oxidation state of the vanadium present in these species arises. Figure 2 shows the e.s.r. spectrum of vanadium (IV), as VO^{2+}, in an aqueous acid solution at 10^{-2} M and the colonial tunicate *E. diaphanes* at 77° K. Similar spectra were observed for *E. molle*, *E. ritteri* and *E. psammion*. The spectra recorded are at different sensitivities and are shown to confirm that vanadium (IV) can be detected in these samples. On the basis of comparisons with standards of known vanadium (IV) concentrations a major fraction of the vanadium present is in the plus-four oxidation state. Care was taken to protect the samples from oxygen, but it cannot be ruled out that the vanadium (IV) was produced from the rapid oxidation of a complex containing vanadium in a lower oxidation state. Although vanadium (III) is only slowly oxidized by oxygen in aqueous, acid solution (Ramsey, Sugimoto and De Vorkin, 1941), certain vanadium (III) complexes in organic solvents (Swinehart, 1971) and aqueous vanadium (II) (Swinehart, 1965) are rapidly oxidized by oxygen. However, the vanadium (III) complexes present in *Phallusia mamillata* (Bielig, Bayer, Dell, Rohns, Mollinger and Rüdiger, 1966) and *Ascidia obliqua* (Lybing, 1954) under acid conditions are slowly oxidized by oxygen. This type of vanadium (III) complex can not be a precursor to the observed vanadium (IV) complex. The question remains as to whether the acid and vanadium are localized in the same cells. Levine (1961) has shown that the "green-cell" blood corpuscles of *Eudistoma ritteri* do not contain acid and do not reduce osmic acid; the latter is a test for the vanadium (III) complexes found in the blood of certain species. Our observations are consistent with the conclusion that vanadium is in the plus four oxidation state in species of the genus *Eudistoma* and consequently may not be susceptible to oxidation by osmic acid.

Observations on a species from the order Stolidobranchia

All other ascidians studied had small vanadium contents or large iron contents compared to other metals present. A study by Carlisle (1958) on the metal content of pooled samples of *Molgula manhattensis* from the order Stolidobranchia shows the presence of vanadium (101 ppm dry weight of flesh without tunic) and niobium (56 ppm dry weight). The vanadium content in this study of the animals without tunic is less than 20 ppm organic dry weight, which is substantially below the level previously reported. This discrepancy may actually be a result of species differences (Monniot, 1969). The iron content of the animal without tunic is 900–1700 ppm organic dry weight. It was noted that freshly collected animals exhibited very large iron content (16,400–26,600 ppm organic dry weight) compared to animals which were kept in a salt water aquarium for several days (900–1700 ppm organic dry weight). The large iron content observed for freshly collected specimens arose from ingested particulate matter from the collection area. A large percentage of the iron in the animals (kept in a salt water aquarium) is localized in the fluid from the heart and the amount of the iron in heart fluid is the same for both freshly collected and aquarium specimens.

Metal contents and evolution of ascidians

Millar (1966) has suggested that the order Aplousobranchiata, Aplousobranchia according to Van Name (1945), be divided into three families. One of the families,

Clavelinidae, is divided into three subfamilies: Clavelininae, Holozoinae and Polycitorinae. The two most developed subfamilies are Holozoinae, which contains the genus *Distaplia*, and Polycitorinae, which contains the genus *Eudistoma*. It seems probable that the adaption of species in the genera *Distaplia* and *Eudistoma* to vanadium occurred independently of species in the order Phlebobranchia and consequently the difference in the oxidation states of vanadium in the different orders is not surprising since the functions of the metals may be different.

It has also been suggested (Webb, 1939) that an early ascidian form had the ability to accumulate vanadium at the expense of other metals, the ability to accumulate vanadium was lost in the two evolutionary lines that developed from this form, and these two evolutionary lines and the accompanying loss of vanadium culminated in the iron containing species of the order Aplousobranchia (*e.g., Amaroucium californicum, A. solidum, Polyclinum planum* and *Synoicum par-fustis*) and Stolidobranchia (*Molgula manhattensis*). Along these evolutionary lines there could be transitional species which have the ability to accumulate both iron and vanadium. The iron contents of *Eudistoma molle* and *Distaplia occidentalis* are of the same order of magnitude as the vanadium. It is possible that these species represent animals which are in transition between the vanadium and iron users.

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Summary

The vanadium and other selected metal contents of primarily California ascidians have been determined. The species *Ascidia ceratodes* and *Perophora annectens* have large vanadium contents as has been predicted for members of the families Ascidiidae and Perophoridae from the order Phlebobranchia. Several species in the order Aplousobranchia have large vanadium contents: the vanadium being present as vanadium (IV) whereas it is vanadium (III) that is found in the order Phlebobranchia. *Molgula manhattensis*, a species from the order Stolidobranchia, shows a large iron content: the metal being localized in the fluid from the heart.

Three dominant fractions were chromatographed from the cells contained in the fluid of *Ascidia ceratodes*. The roles of the compounds present in these fractions are discussed. The spectra of these fractions are correlated with the spectrum of the cells.

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